(R4)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/GB2004/002994	International filing date *(day/month/year)* 12.07.2004
Priority date *(day/month/year)* 11.07.2003	
International Patent Classification (IPC) or both national classification and IPC A61K47/48	
Applicant POLYTHERICS LIMITED	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/002994

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/002994

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-14 (all partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1-14 (all partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	12(partially)
	No:	Claims	1-11, 13-14 (all partially)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14 (all partially);
Industrial applicability (IA)	Yes:	Claims	1-14 (all partially)
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The present claims 1 and 12 relate to an extremely large number of compounds. Support (and disclosure) in the sense of Art. 6 PCT is to be found however for only very small proportion of compounds claimed, see p. 22 and examples 1-7. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 1 and 12 (PCT Guidelines 9.19 and 9.23).

The search of claims 1 and 12 was restricted to those claimed compounds which appear to be supported and a generalisation of their formulae, namely the compounds according to claim 1 wherein the polymer is any polymer, z1 and z2 are any biological molecule and the linker is any of those described in the examples 1-7 and on p. 22 (claims 1-14 (partially)).

2. The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1: WO 99/45964 A

D2: ROBERTS M J ET AL: "Chemistry for peptide and protein PEGylation" ADVANCED DRUG DELIVERY REVIEWS, AMSTERDAM, NL, vol. 54, no. 4, 17 June 2002 (2002-06-17), pages 459-476, XP002293146 ISSN: 0169-409X

D3: WILBUR D SCOTT ET AL: "Monoclonal antibody Fab' fragment cross-linking using equilibrium transfer alkylation reagents. A strategy for site-specific conjugation of diagnostic and therapeutic agents with F(ab')-2 fragments" BIOCONJUGATE CHEMISTRY, vol. 5, no. 3, 1994, pages 220-235, XP002313937 ISSN: 1043-1802

D4: WO 01/05818 A

D5: US 2002/103259 A1

D6: US-A-5 786 387

D7: DEL ROSARIO R B ET AL: "SULFHYDRYL SITE-SPECIFIC CROSS-LINKING AND LABELING OF MONOCLONAL ANTIBODIES BY A FLUORESCENT EQUILIBRIUM TRANSFER ALKYLATION CROSS-LINK REAGENT" BIOCONJUGATE CHEMISTRY, vol. 1, no. 1, 1990, pages 51-59, XP002313938 ISSN: 1043-1802

D8: LIBERATORE F A ET AL: "SITE-DIRECTED CHEMICAL MODIFICATION AND CROSS-LINKING OF A MONOCLONAL ANTIBODY USING EQUILIBRIUM TRANSFER ALKYLATING CROSS-LINK REAGENTS" BIOCONJUGATE CHEMISTRY, vol. 1, no. 1, 1990, pages 36-50, XP002313939 ISSN: 1043-1802

D9: WO 99/55377 A

D10: BROCCHINI ET AL.: "Molecular yardsticks. Synthesis of extended equilibrium transfer alkylating cross-link reagents and their use in the formation of macrocycles" JOURNAL OR THE AMERICAN CHEMICAL SOCIETY, vol. 110, 1988, pages 5211-5212, XP002313940

3. The present application is directed to polymer-protein conjugates which comprise one of the linkers described in the examples (p. 22 and examples 1-7).

4. Novelty (Art. 33(2) PCT)

D1discloses PEG-protein compounds bound via formation of two proximal bonds of amide nature (passages cited in the SR). Therefore, the present subject-matter is novel over D1.

D2 discloses general methods for binding proteins and PEG via disulfide bonds (Fig. 6) as well as PEG structures having two free functional groups (Fig. 14). However, PEG structures having two free functional groups that could form two disulfide bonds with the proteins are not disclosed. Therefore, the present subject-matter is novel over D2.

D3 discloses trifunctional cross-linking reagents capable of binding a polymeric structure with two proteins (antibodies). Among the trifunctional cross-linking reagents, the compounds 1a and 1b are disclosed (see Fig .2). Said compounds are the same as some of the present application. Therefore, the subject-matter of present claim 1 is not novel over D3.

D4 discloses PEG-cyclosporin compounds (see claim 1). This compounds do not encompass the formation two disulfide bonds between cyclosporin and PEG. Therefore, the present subject-matter is novel over D4.

D5 (see compounds cited in the SR) disclose terminally-branched polymeric linkers between PEG and biological compounds. However, the binding is not via a disulfide bond and the biological compound is not a protein. Therefore, the present subject-matter is novel over D5.

D6 discloses lipid double-chain derivatives containing PEG (see passages cited in the SR) by means of a terminally branched linker. The binding between the PEG and the lipid part is not via a disulfide bond and the biological compound is not a protein but a lipid. The present subject-matter is therefore novel over D6.

D7 and D8 disclose the cross-linking of antibodies via branched linkers according to the present application (see SR). However, neither D7 nor D8 disclose PEG-protein conjugates. The present subject-matter is thus novel over D7 and D8.

D9 discloses PEG-IFN conjugates bound via a disulfide bond (see SR). However, this linker is a branched linker. D9 does not destroy the novelty of the present subject-matter.

D10 discloses further compound suitable for cross-linking proteins. The compounds of D10 have been used in the present application.

Sumarizing, the compounds of present claim 1 are not novel over the content of D3, however, the compounds of formula II and III are novel over D1-D10.

5. Inventive step (Art. 33(3) PCT):

The subject-matter of claim 1 is not novel over D3. Therefore, it cannot be considered to be inventive.

Furthermore, the skilled person could combine the teaching of D3 with the trifunctional linkers of D7, D8 or D10 to provide further conjugates according to the present application.

When the polymer is PEG, the skilled person could combine the D3, D7, D8 or D10 with the teaching of D2 which discloses in Fig. 1 the PEG-derivatives suitable for binding to amines (for instance, those of D3, D7, D8 or D10). Therefore, compounds according to present claim 1 could be obtained.

The compounds of present claim 12 should be considered also not inventive, since D2 taught the skilled person PEG-derivatives that bind to the amines of D3, D7, D8 or D10.

6. Industrial applicability (Art. 33(4) PCT).

The present subject-matter is industrially applicable.

Re Item VII
Certain defects in the international application

7. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1-D10 mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

8. Present claim 12 is unclear (Art. 6 PCT), since it is dependent upon the compounds of claims 10 or 11, which are however process claims.